

Risk Declaration

For further instructions on how to write your risk declaration, see SOP on the lifebio server.

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Date: 2016-06-14

Experiment: iGEM project

1. Description of experiment:

Short and precise, explaining the different steps in your experiment. If you are working with microorganisms, remember to specify the species and the biosafety level required.

Genomic extraction of genomic DNA from *Escherichia coli* (*E. coli*) (Biosafety level 1), *Synechocystis* sp. PCC6803 (Biosafety level 1), *Yarrowia lipolytica* (*Y. lipolytica*) (Biosafety level 1), *Bacillus subtilis* (*B. subtilis*) (Biosafety level 1) and *Saccharomyces cerevisiae* (*S. cerevisiae*) (Biosafety level 1).

Amplification of different DNA parts by PCR. Enzyme digestion and ligation. Piecing together constructs using Gibson Assembly. Transformation of plasmids/linear DNA fragments into *E. coli*, *Synechocystis* sp. PCC6803, *Y. lipolytica*, *B. subtilis* and *S. cerevisiae*.

Co-cultivation trials in BG11 media with *Synechocystis* and 1 of the 4 other organisms. *Synechocystis* will produce the carbon source for the other microorganism by fixating CO₂, while *Synechocystis* in return gets an amino acid from the other organism (we'll make the cyanobacteria auxotrophic). The other organism will also produce some product, resulting in a production system which does not require a carbon source (except CO₂).

Cell counting through flow cytometry to obtain the ratio of the different species in the co-culture. Quantification of produced product through for example HPLC. Promoter study in *S. cerevisiae* with acetate as the only carbon source (with a fluorescent protein as the reporter).

2. KLARA Risk Assessments read:

Specify risks assessments that are relevant to your experiment. Use the information when you summarize the risks and how to minimize them under sections 4 and 5.

SB/IB-Dishwasher

SB/IB-Thermal Cycler (PCR)

SB/IB-Rotary Shakers / Incubators

SB/IB-Rotary Shakers / Incubators

SB/IB-bench top autoclave

SB/IB - -80 degree freezer

SB/IB-Heat block

SB/IB-Waterbath

SB/IB-Centrifuge (High speed) Avanti J-20 XP

SB/IB-Centrifugal vacuum vaporators

SB/IB-Sterile work/LAF-bench

SB/IB-Gel electrophoresis

SB/IB-Vertical autoclave

SB/IB-Ultracentrifuge Beckman LE80K










SB/IB-Centrifuge Sigma 4K15






SB/IB-small centrifuges











SB/IB-Gel electrophoresis w GelRed

















3. Chemicals:







Specify MSDS read and safety information for each chemical in your experiment. For every chemical, specify the chemical name, CAS-number, the concentration of the final solution (if applicable), CLP hazard pictogram(s) (use table below) and hazard statement(s).

CLP hazard pictograms in accordance to EG 1272/2008								
								
Gas under pressure	Explosive	Oxidizing	Flammable	Corrosive	Health hazard	Acute toxicity	Serious health hazard	Hazardous to the environment

Chemical name and [CAS-No]	Conc. of handled solution	Pictogram(s)	H statement(s)
Ethanol (64-17-5)	95-70 %	 	H225 Highly flammable liquid and vapour H319 Causes serious eye irritation
Tris-HCl (1185-53-1)		None	None
EDTA disodium salt dihydrate (6381-92-6)	1 g/L	 	H332 Harmful if inhaled H373 May cause damage to organs through prolonged or repeated exposure
Lithium acetate (6108-17-4)	1M		H319 Causes serious eye irritation
Agarose (9012-36-6)	1%	None	None
GelRed Nucleic Acid Stain, 10,000X in water		None	None
GelGreen		None	None

Ampicillin (69-52-3)	Stock: 100 mg/mL. Working solution: 100 µg/ml		H317 May cause an allergic skin reaction H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled
Tryptone (91079-40-2)		None	None
Yeast extract (8013-01-2)	10 g/L	None	None
Calcium chloride dihydrate (10035-04-8)	0.1 M		H319 Causes serious eye irritation
D(+)-Glukos (50-99-7)	20 g/L	None	
Chloramphenicol (56-75-7)	Stock: 25 mg/mL. Working solution: 25 µg/ml		H350 May cause cancer
Spectinomycin	Stock: 50 mg/mL. Working solution: 50 µg/mL	None	None
Kanamycin sulfate, mixture of Kanamycin A (main component) and Kanamycin B and C (70560-51-9)	Stock: 50 mg/mL. Working solution: 50 µg/mL		H360 May damage fertility or the unborn child
G418 disulfate salt solution (Klara id: 43619)	Stock: 200 mg/mL. Working solution: 200 µg/mL		H317 May cause an allergic skin reaction H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled
di-Potassium hydrogen phosphate anhydrous (7758-11-4)	30.6 g/L	None	None
Magnesium sulfate heptahydrate (10034-99-8)	75 g/L	None	None
Citric acid (77-92-9)	6 g/L		H319 Causes serious eye irritation
Ammonium iron(III) citrate (1185-57-5)	6 g/L		H315 Causes skin irritation H319 Causes serious eye irritation H335 May cause respiratory irritation
Sodium carbonate (497-19-8)	20 g/L		H319 Causes serious eye irritation
Boric acid (10043-35-3)	2.86 g/L		H360FD May damage fertility or the unborn child
Manganese(II) chloride tetrahydrate (13446-34-9)	1.81 g/L		H302 Harmful if swallowed H411 Toxic to aquatic life with long-lasting effects

Zinc sulfate heptahydrate (7446-20-0)	222 mg/L	  	H302 Harmful if swallowed H318 Causes serious eye damage H410 Very toxic to aquatic life with long-lasting effects
Sodium molybdate dehydrate (10102-40-6)	390 mg/L	None	None
Copper(II) sulfate pentahydrate (7758-99-8)	79 mg/L	 	H302 Harmful if swallowed H315 Causes skin irritation H319 Causes serious eye irritation H410 Very toxic to aquatic life with long-lasting effects
Cobalt(II) nitrate hexahydrate (10026-22-9)	49.4 mg/L	   	H272 May intensify fire; oxidizer H302 Harmful if swallowed H317 May cause an allergic skin reaction H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled H341 Suspected of causing genetic defects H350i May cause cancer H360F May damage fertility or the unborn child H400 Very toxic to aquatic life H410 Very toxic to aquatic life with long-lasting effects
Sodium nitrate (7631-99-4)	1.5 g/L	 	H272 May intensify fire; oxidizer H302 Harmful if swallowed H315 Causes skin irritation H319 Causes serious eye irritation H335 May cause respiratory irritation
Sodium hydrogen carbonate (144-55-8)	50 mM	None	None
Agar (9002-18-0)	15 g/L	None	None
HEPES (7365-45-9)	11.9 g/L	None	None
L-Arginine (74-79-3)	5 mM		H319 Causes serious eye irritation
L-Glutamine (56-85-9)	5 mM	None	None
Phenol (108-95-2)		   	H301 Toxic if swallowed H311 Toxic in contact with skin H314 Causes severe skin burns and eye damage H331 Toxic if inhaled H341 Suspected of causing genetic defects H373 May cause damage to organs through prolonged or repeated exposure H411 Toxic to aquatic life with long-lasting effects

Chloroform (67-66-3)			H302 Harmful if swallowed H315 Causes skin irritation H319 Causes serious eye irritation H331 Toxic if inhaled H336 May cause drowsiness or dizziness H351 Suspected of causing cancer H361d Suspected of damaging the unborn child H372 Causes damage to organs through prolonged or repeated exposure
Sodium dodecyl sulfate (151-21-3)	1%		H315 Causes skin irritation H319 Causes serious eye irritation
Sodium acetate (127-09-3)		None	None
Dimethyl sulfoxide, DMSO (67-68-5)		None	H227 Combustible liquid.
Natriumtiosulfat (7772-98-7)		None	None
Erytromycin (114-07-8)	Stock: 1 mg/mL. Working solution: 1 µg/mL	 	H317 May cause an allergic skin reaction. H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
Tetracycline hydrochloride (64-75-5)	Stock: 10 mg/mL. Working solution: 10 µg/mL		H315 Causes skin irritation. H319 Causes serious eye irritation. H335 May cause respiratory irritation.
Uracil (66-22-8)	0.06 g/L	None	None
Ammonium chloride (12125-02-9)	0.5345 g/L		H302 Harmful if swallowed. H319 Causes serious eye irritation.
Ammonium sulfate (mass) (7783-20-2)	2 g/L	None	None
Potassium dihydrogen phosphate anhydrous (7778-77-0)	60 g/L	None	None
di-Potassium hydrogen phosphate anhydrous (7758-11-4)	140 g/L	None	None

3.1 Use of restricted chemicals

Use the chemical information in KLARA to answer the following questions. (In KLARA you will find this information listed under the section "Regulations" or in Swedish "Regler och krav". Note! If your chemical does **not** have a classification, this section will not show up on the KLARA information page.)

- a) Are any of the chemicals classified as either a Group A or Group B chemical? If yes, which one(s), and do we have a valid permit?

No.

b) Are any of the chemicals classified as a CMR (Carcinogenic, Mutagenic or Reprotoxic) substance? If yes:

i. Which one(s)?

- i. Chloramphenicol (56-75-7)
- ii. Kanamycin sulfate, mixture of Kanamycin A (main component) and Kanamycin B and C (70560-51-9)
- iii. Boric acid (10043-35-3)
- iv. Cobalt(II) nitrate hexahydrate (10026-22-9)

ii. How frequently will you be handling them (times/month)?

- i. Regularly. Will be dissolved in liquid, which greatly reduces the risks.
- ii. A few times. Will be dissolved in liquid, which greatly reduces the risks.
- iii. Used once to make a large diluted stock solution.
- iv. Used once to make a large diluted stock solution.

c) Does any of the chemicals have the hazard statement H317 and/or H334? If yes:

i. Which one(s)?

- i. Ampicillin (69-52-3).
- ii. Cobalt(II) nitrate hexahydrate (10026-22-9)
- iii. G418 disulfate salt solution (Klara id: 43619)
- iv. Erytromycin (114-07-8)

ii. How frequently will you be handling them (times/month)?

- i. Regularly. Will be dissolved in liquid, which greatly reduces the risks.
- ii. Used once to make a large diluted stock solution.
- iii. A few times. Will be dissolved in liquid, which greatly reduces the risks.
- iv. A few times. Will be dissolved in liquid, which greatly reduces the risks.

iii. Do you have any allergies?

No.

4. Comments on risks:

Identify and specify risks associated with reactions or combinations of chemicals, equipment used or other potential risks. Where is the actual element of risk? When do you need to take precautions to work in a safe way?

Ethanol: Flammable, do not use close to open flame.

EDTA: Avoid inhalation

Lithium acetate: Avoid contact with eyes.

GelRed: Avoid inhalation of vapor or mist. Avoid direct contact with substance (binds to DNA).

GelGreen: Avoid inhalation of vapor or mist. Avoid direct contact with substance (binds to DNA).

Ampicillin: Avoid inhalation and contact with skin.

Chloramphenicol: May cause cancer. Avoid inhalation and contact with skin.

Calcium chloride dihydrate: Avoid contact with eyes.

Kanamycin sulfate: Avoid inhalation and contact with skin.

G418 disulfate salt solution: Avoid breathing dust/fume/gas/mist/vapours/spray. Avoid contact with skin.

Erytromycin: Avoid breathing dust/fume/gas/mist/vapours/spray.

Wear protective gloves/protective clothing/eye protection/face protection.

If experiencing respiratory symptoms: Call a POISON CENTER/doctor

Tetracycline hydrochloride: Avoid breathing dust/fume/gas/mist/vapours/spray.

IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Ammonium chloride: Wash ... thoroughly after handling

Wear protective gloves/protective clothing/eye protection/face protection.

IF SWALLOWED: Call a POISON CENTER/doctor/... if you feel unwell.

IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

If eye irritation persists: Get medical advice/attention.

Citric acid: Avoid contact with eyes.

Ammonium iron(III) citrate: Avoid breathing dust/fume/gas/mist/vapours/spray. Avoid contact with eyes.

Sodium carbonate: Do not breathe dust/fume/gas/mist/vapours/spray. Avoid contact with skin and eyes.

Boric acid: Avoid breathing dust. Avoid contact with skin and eyes.

Manganese(II) chloride tetrahydrate: Don't swallow. Avoid release into environment.

Zinc sulfate heptahydrate: Avoid release into the environment. Use contact with eyes and skin.

Copper(II) sulfate pentahydrate: Avoid release to the environment. Avoid contact with skin and eyes. Harmful if swallowed.

Cobalt(II) nitrate hexahydrate: Keep away from heat. Avoid breathing dust/fume/gas/mist/vapours/spray. Avoid contact with skin and eyes. Harmful if swallowed. Skin penetrating substance.

Sodium nitrate: Harmful if swallowed. Avoid breathing dust/fume/gas/mist/vapours/spray. Avoid contact with eyes.

L-Arginine: Avoid contact with eyes.

Phenol: Do not breathe dust/fume/gas/mist/vapours/spray. Avoid contact with skin and eyes. Skin penetrating substance.

Chloroform: Avoid contact with skin and eyes. Avoid inhalation

Sodium dodecyl sulfate: Avoid contact with skin and eyes and avoid inhalation. Must not be exposed to heat, flame or sparks.

DMSO: Keep away from heat.

When running the centrifuges they should be equally balanced.

Handle gel electrophoresis with care, electric current is used.

Autoclaves: hot surfaces, use heatgloves when removing autoclaved equipment.

Heatblock: do not touch hot surface.

5. Risk reductions:

5.1 Storage:

Some chemicals can be hazardous if they are not kept in a proper way (e.g. flammable compounds). Specify how you will store those chemicals safely.

Ethanol: Store in cool place. Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage.

Cobalt(II) nitrate hexahydrate and similar toxic substances: Keep containers tightly closed in a dry, cool and well-ventilated place marked with appropriate hazard pictograms.

Sodium dodecyl sulfate: Keep containers tightly closed in a dry, cool and well-ventilated place.

5.2 Chemical handling:

Specify how to minimize the risks in handling the chemical(s), (e.g. use of fume hood, ventilation arms, and which type of gloves you need to use).

Personal protection needed:

- ☒ Gloves and lab coat
- ☒ Safety glasses
- ☐ Facial mask
- ☐ Other, specify: ventilation arm

For a majority of the work only the most basic protective gloves (grey) will be needed, the exception is when working with hazardous compounds, antibiotics or DNA staining (which will require orange gloves).

Hazardous compounds (especially in powder form) will be handled in a fume hood while wearing protective gloves (orange).

Red gloves and fume hood will be used when handling chloroform, cobalt(II) nitrate hexahydrate, and phenol.

Zinc sulfate heptahydrate (7446-20-0) will only be handled while wearing protective eye- or facewear.

5.3 Waste handling:

Specify what kind of waste is produced, and how it is handled/disposed of. Consider every step in your experiment. Remember that you will likely generate both solid and liquid waste. If you are disposing of biological waste containing antibiotics, check and state whether or not the antibiotic is inactivated during autoclavation.

All waste that has been in contact with biohazard samples need to be autoclaved. All garbage, materials and liquids that potentially are biohazards will be thrown in a special garbage bag that will be autoclaved. Liquid biowaste is collected in plastic container and autoclaved before poured in the drain.

Materials that have been in contact with biohazard will be rinsed twice with 70% ethanol (also collected in plastic container) and placed in dishwasher.

Agar plates will be disposed in marked boxes.

Chemical waste will be collected in waste containers, and labeled with contents, name and date. The chemical waste is then handed over to Stena Recycling for destruction. Ampicillin, kanamycin and spectinomycin will be collected and handled as chemical waste. If volume is less than 5L, the container will be given directly to Stena Recycling (labeled as "GMO"). If the volume is more than 5L, it will first be autoclaved and then given to Stena Recycling.

Solutions containing Lithium ions should not be poured into the sink, instead they should be collected and handed over to Stena Recycling.

6. Final evaluation of risks

Take into consideration the probability of an accident occurring and the severity of the possible consequences to evaluate the risk of your experiment (see evaluation matrix in SOP).

Choose one of the following:

- ☒ Acceptable risk
- ☐ Some risk
- ☐ Severe risk
- ☐ Very severe risk

I declare that I have read the Risk Assessments and MSDS stated above and that I am aware about the risks involved with this experiment. I will follow the guidelines concerning safety precautions to minimize the risks associated with this experiment.

Signature

Signature

Signature

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The risk declaration has been read by:

Signature of Research Engineer/Lab manager